Melon Fly (Diptera: Tephritidae) Genetic Sexing: All-male Sterile Fly Releases in Hawaii

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Abstract. The first practical genetic sexing strain for the melon fly, Bactrocera cucurbitae, developed in Hawaii was mass-reared and released as sterile males into wild fly populations. Significant improvements in the field quality of sterile males were made with the pupal color strain in which males can be separated from females on the basis of pupal coloration using photoelectric sorting machines. Earlier, quality control tests indicated that the strain mass-rears adequately, and is very competitive with wild flies based on field cage studies of mating ability and survival. Open field studies were conducted between 2002 and 2004 on three Hawaiian islands in increasingly larger test areas, and with increasing numbers of sterile males released (up to 1,500,000/wk). Results indicated that the sexing strain significantly impacted the wild population, causing high, induced sterility up to ca. 75% in both residential and commercial vegetable growing areas of Hawaii. The field tests have shown that the sexing strain is worthy of mass production and release in large-scale melon fly SIT programs.

The melon fly, *Bactrocera cucurbitae* (Coquillett), is a serious economic pest of fruits and vegetables, especially cucurbits, in Asia and the Pacific (White and Elston-Harris 1992). In recent decades, biological control techniques, especially the sterile insect technique (SIT), have gained wider use due to their environmentally benign nature. Large-scale control or eradication programs utilizing the SIT against the melon fly were successful in the Pacific basin, including Japan, where the melon fly was eradicated (Kakinohana et al. 1990, Hibino and Iwahashi 1991). One important factor in promoting the use of the SIT against tephritid pests has been the development of effective genetic sexing strains that permit the separation of males from females at some developmental stage (Robinson et al. 1999). By releasing only sterile males, the efficiency of the technique can be increased several fold, and fruit damage due to stings by sterile females is avoided (McInnis et al. 1994, Rendon et al. 2000).

The first genetic sexing strain for the melon fly, *Bactrocera cucurbitae*, based on pupal coloration was developed and evaluated on a small scale (McInnis et al. 2004). These initial tests determined that the melon fly pupal color sexing strain was fully compatible with wild melon flies, including mating and survival abilities of sterile lab-reared males in field cages. Encouraging results from these preliminary tests led to a series of field experiments, reported here for the first time, involving releases of sterile males-only flies in several melon fly infested areas of Hawaii. Field trials were carried out on three major islands in the Hawaiian chain, first on the island of Hawaii (2002), then on Maui (2003), and finally on Oahu (2004). These studies were integrated into the ongoing USDA/ARS Area-Wide IPM program against the four pest species of tephritid fruit flies in Hawaii.

Materials and Methods

Following the encouraging initial small-scale rearing and field cage studies (McInnis et al. 2004), the melon fly sexing strain was expanded by mass-production and prepared for sterile fly release into wild fly infested areas. We began a program to integrate the new sexing strain into the ongoing IPM program against the melon fly on the Big Island of Hawaii. Flies were reared to pupation at the USDA/ARS/PBARC laboratory in Honolulu HI, then pupal color sorted using high speed photoelectric sorting machines. At ca. 2 days prior to emergence the all-male pupae were dyed with a standard fluorescent dye to mark emerging adults, then shipped inter-island via air cargo to the field test site, except for Oahu. The pupae were dispensed into 2 gallon "chicken" buckets containing ca. 1100 pupae/bucket and held with food and water (agar) for ca. 5 days under ambient conditions in large holding rooms prior to ground release in the field. Figure 1 shows the test site on the Big Island consisting of an 8 X 5 km (40 sq. km) grid and the outlined 4 X 5 km (20 sq. km) grid where sterile flies were released. Flies were initially released at six sites on the ground at the top right corner of the grid, mostly in a residential area, then gradually were expanded further into the inner 4 X 5 release grid over ca. 6 months of releases. Approximately 150,000–200,000 males were released once per week into the grid. The important sterile:wild fly overflooding ratios were monitored weekly by standard cuelure bucket traps covering the release area.

Statistical analysis of the correlations between the sterile:wild fly ratios and the observed egg sterility at different time periods (see Table 1) was performed (Snedecor and Cochran 1967). In addition, the numbers of hatched and unhatched eggs for control and fly release sites were compared for each sampling period and the probabilities of obtaining such results by chance were provided by Chi-Square analysis.

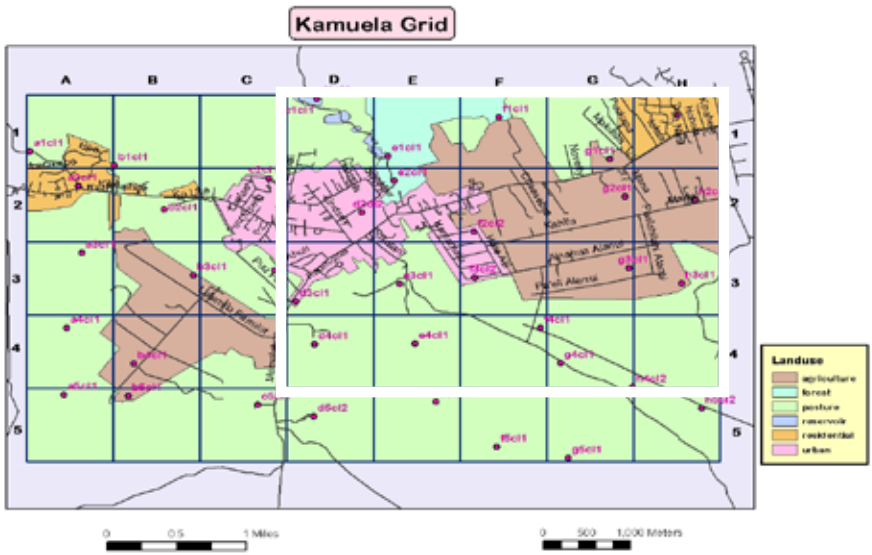

Figure 1. Sterile melon fly release area for the genetic sexing strain in the mostly residential but partly agricultural Kamuela area, island of Hawaii, in 2002. Total grid size is 40 sq. km. (8 X 5 km) with initial fly release area (inner grid) of 20 sq. km. (4 X 5 km).

Table 1: Melon fly egg sterility data for periods before and after SIT releases of the genetic sexing strain in control and treated areas on the island of Hawaii, 2002.

Area		Total no. eggs	No. hatched	No. unhatched	% sterility	Chi-Sq. Prob.[1]
Pre-Release (Oct. '01–Jan.'02)		2428	2171	257	10.6	
Months Post-Release						
1	Control	75	54	21	28.0	93.8 (P<0.001)
	Treated	131	28	103	78.6	
	Control	39	35	4	10.3	63.4 (P<0.001)
	Treated	281	69	212	75.4	
3 and 4	Control	72	65	7	9.7	79.5 (P<0.001)
	Treated	108	23	85	78.7	
5 and 6	Control	164	112	52	31.7	44.8 (P<0.001)
	Treated	128	36	92	71.9	
7 and 8	Control	113	98	15	13.3	152.1 (P<0.001)
	Treated					
8 Months	Control	463	364	99	21.3	403.4 p<0.01)
Cum. Total						
	Treated	**802**	**172**	**630**	**78.6**	

[1] Chi.- Square analysis comparing the numbers of hatched and unhatched eggs for Control and Treated (release) sites for each sampling period**.** The probabilities of obtaining such results by chance are provided (these are highly significant for all sampling periods, P< 0.001).

Results and Discussion

The control and release areas overlapped in wild flies per trap-day measures until fly releases began in Feb., 2002. After that time, the control area always had a higher wild fly per trap day catch, and frequently was ca. 5–10 times higher than for the treated area. It should be noted that the control area had other IPM strategies taking place, including bait sprays, male annihilation, and field sanitation, as was true for the treated area, but only the treated area received sterile flies. After August, 2002, the fly releases were expanded to include the former control area so there was no true fly-free control to provide a comparison.

The critical measure of success of an SIT program is the level of induced egg sterility one obtains from released sterile flies. The results of egg dissections in both treated and control areas can be seen in Table 1. Fly releases were conducted for 8 months before the numbers of eggs obtained were so small as to be meaningless, plus the expansion of sterile flies into the former control area, outside the inner 4 X 5 km grid, made the control vs. treated area comparison of dubious value. Based on Chi-Square comparisons of the number of hatched and unhatched eggs for control and release sites, the release area had significantly higher egg sterilities even after only 1 month of releases, reaching ca. 75% sterility, or higher, at all times during the test ($P<0.001$). Control egg sterilities were 11% before releases, and averaged ca. 15% during the releases.

Due to the success of the open field fly releases using the melon fly sexing strain, the USDA/ARS laboratory of the senior author, in collaboration with the University of Hawaii (Manoa) and the co-authors, began releasing sterile melon flies on the island of Maui, again as part of the current IPM program. Fly releases began in March, 2003, in a ca. 10 sq km area in lower Kula, Maui, where a much larger melon fly population existed compared to the earlier Big Island population. This area greatly challenged the SIT capability of the new strain, and required a large expansion of our mass-rearing production in order to succeed. Production of sterile flies increased from ca. 200,000/wk to ca. 800,000/wk. for this test on Maui. In addition, in order to produce large numbers of flies at a consistently high level of purity, we adopted a filter rearing system in which a purified colony is continuously maintained then expanded through 3 cycles of rearing, in order to produce sufficient numbers for color machine sorting of pupae and release of virtually 100% males into the field. Results of the sterile fly releases in Maui between March and September, 2003 are shown in Figure 2. As can be noted in the figure, egg sterility rose significantly once the sterile: wild (S:W) fly ratio increased significantly in July, 2003, after 4 months of releases. As the S:W ratio increased into August, the egg sterility obtained from egg dissections from host fruit collected in the field test site also continued to increase. Finally, when the fly releases were discontinued in September, 2003, the egg sterility dropped accordingly until the final collections made in November. The overall statistical correlation between the S:W fly ratio and egg sterility was highly significant ($r = 0.850$, $P< 0.01$).

Encouraged by these results, we proceeded to attempt sterile fly releases on the island of Oahu, HI in a large commercial plantation covering ca. 450 ha and many types of melon fly hosts throughout the year. We increased our fly production to 1,500,000 sterile males per week from a total production of ca. 5 million pupae prior to color sorting. During the period from Nov., 2003 to July, 2004 we released flies on the standard weekly basis and trapped flies on the same 2-week interval. Unfortunately, the wild fly population level was so high that the S:W overflooding ratio was less than 1:1 until the 5th month of releases. Egg sterility increased slowly in accordance with the S:W ratio, reaching ca. 45% at a ca. 1.5:1 ratio. Then, after releases stopped in early July, both the S:W ratio and egg sterility dropped until all sterile flies died off in the field some 2 months later. The relationship between the S:W ratio and egg sterility was again statistically significant, $r = 0.934$, $P < 0.05$.

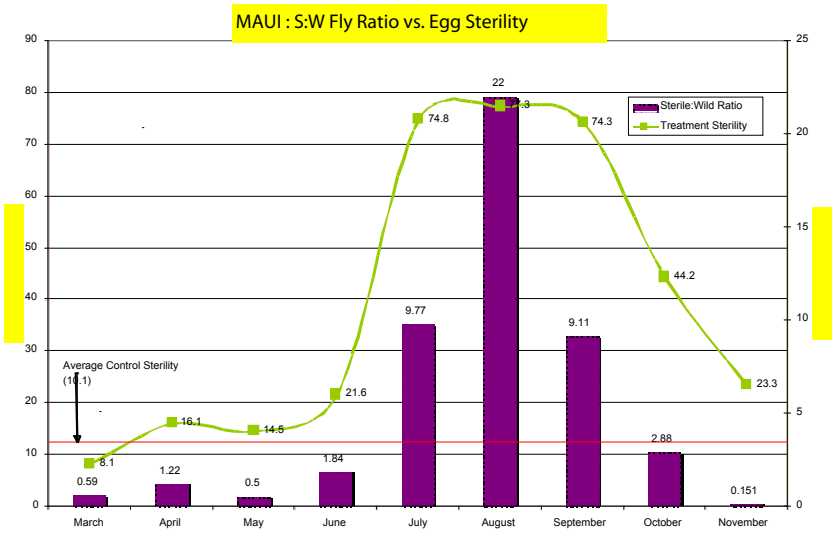

Figure 2. Egg sterility vs. sterile: wild fly ratios for the melon fly sexing strain on Maui during March–September, 2003.

In conclusion, the newly developed melon fly sexing strain, has proven to be a very competitive strain, as evidenced by earlier results indicating high quality laboratory and field cage performances. The following SIT programs for this strain progressed over three islands and three years from 2001 to 2004 in increasingly larger test sites and larger wild fly populations. Fly production increased to compensate for the higher wild fly populations, until the Oahu program when the S:W ratio never exceeded 1.5:1, in spite of maximal sterile male production. Nonetheless, the egg sterility obtained in all three programs was relatively high, indicating very good competitiveness for the strain under field conditions. Based on these studies, the strain is ready for both mass production and aerial releases of sterile males into the field. Aerial releases will likely improve fly distribution and, consequently, melon fly control by the SIT.

Acknowledgements

The authors would like to thank the technical staff at the USDA/ARS locations in Honolulu and Kamuela in Hawaii, and the University of Hawaii technical personnel and students in Honolulu and Maui, who assisted in the rearing and field operations of the project on several islands. We also thank Todd Shelly for his critical review of an earlier version of this paper.

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